

***Lembosia mimusopis* sp. nov. from Thailand**

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ABSTRACT—A novel species *Lembosia mimusopis* is introduced with evidence from morpho-molecular characterization. It was collected from the leaves of *Mimulus elengi* in Chiang Rai Province, Thailand. The new species is unique in having a mucilaginous sheath surrounding its immature ascospores. LSU sequence analyses phylogenetically support separation of this species from other *Lembosia* species.

KEYWORDS—Asterinaceae, Asterinales, Dothideomycetes, Lembosiaceae, Sapotaceae

Introduction

Léveillé (1845) established *Lembosia* with four species: *Lembosia tenella* Lév. (type species), *L. dendrochili* Lév., *L. drimydis* Lév., and *L. macula* Lév.

Hansford (1946) included *Lembosia* in his new family *Asterinaceae* Hansf. Based on morphology, Hosagoudar & al. (2001) and Hosagoudar (2012) excluded *Lembosia* from *Asterinaceae* and treated the genus as the type of *Lembosiaceae* Hosag. However, Hongsanan & al. (2014) re-instated *Lembosia* within the *Asterinaceae* based on molecular data derived from *L. albersii* Henn. Dai & al. (2018) revealed that two species, *L. albersii* and *L. xyliae* Zeng & al., are phylogenetically separate from *Asterinaceae* but retained them in the same order, *Asterinales*. Three other species (*L. syzygii* Sivan. & R.G. Shivas, *L. abaxialis* Firmino & R.W. Barreto, *L. brigadeirensis* Firmino & al. clustered with *Asterina* species in a distinct clade and Dai & al. (2018) treated them in *Asterinales* sensu lato. There are about 200 *Lembosia* species epithets listed in Index Fungorum (2019). The type species *L. tenella* has not yet been sequenced, and only five *Lembosia* species have been sequenced.

Lembosia species are considered as host-specific on plants in tropical and sub-tropical regions and are usually identified based on association with their host family or genus (Müller & von Arx 1962, Sivanesan & Shivas 2002). For instance, Sivanesan & Shivas (2002) introduced *L. syzygii* from the leaves of *Syzygium suborbiculare* (Benth.) T.G. Hartley & L.M. Perry and *Araucaria heterophylla* (Salisb.) Franco. The inherent morphological characters of *Lembosia* are oval, elongated thyriothecia with an X- or Y-shaped or longitudinal dehiscence, lateral appressoria on the hyphae, and ovate to globose asci containing 4–8 brown uniseptate ascospores (Hosagoudar & Goos 1991, Hosagoudar 2012). The asexual morph of this genus is undetermined.

In this study, we introduce a novel species, *Lembosia mimusopsis*, which was collected from the leaves of *Mimusops elengi* in Thailand. Morphological characters of the new taxon and its taxonomic placement supported by phylogenetic analysis are provided.

Materials & methods

Sample collection, morphological studies, specimen deposition

Fresh leaf specimens were collected from Mae Fah Luang Botanical Gardens, Chiang Rai, Thailand, in December 2018. Specimens were examined using a Motic SMZ 168 Series microscope. Hand sections of the fruiting structures were mounted in water and 5% KOH for microscopic studies and photomicrography. Blue ink was used to stain the mucilaginous sheath around the ascospores. The micro-morphology was examined using a Nikon ECLIPSE 80i compound microscope and photographed using a Canon 750D digital camera fitted to the microscope.

Measurements were made with the Tarosoft (R) Image Frame Work program and figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. Specimens were deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU). The new taxon was linked with Facesoffungi and Index Fungorum databases as explained in Jayasiri & al. (2015) and Index Fungorum (2019).

TABLE 1. Taxa used in the phylogenetic analyses and their GenBank accession numbers and voucher numbers

SPECIES	VOUCHER	GENBANK (LSU)
<i>Asterina cesticola</i>	TH 591	GU586215
<i>Asterina chrysophylli</i>	VIC 42823	KP143738
<i>Asterina cynometrae</i>	MFLU 13-0373 [T]	NG057120
<i>Asterina fuchsiae</i>	TH 590	GU586216
<i>Asterina magnoliae</i>	MFLU 16-0072	MG844186
<i>Asterina melastomatis</i>	VIC 42822 [T]	NG057055
<i>Asterina phenacis</i>	TH 589	GU586217
<i>Asterina siphocampyli</i>	PPMP 1324	HQ701140
<i>Asterina weinmanniae</i>	TH 592	GU586218
<i>Asterina zanthoxyli</i>	TH 561	GU586219
<i>Asterotexis cucurbitacearum</i>	PMA M-0141224 [T]	HQ610510
<i>Batistinula gallsiae</i>	VIC 42514	KP143736
<i>Buelliella physciicola</i>	Ertz 18113	KP456147
<i>Buelliella poetschii</i>	Ertz 18115	KP456149
<i>Hemigrapha atlantica</i>	Ertz 14014	KP456151
<i>Inocyclus angularis</i>	VIC 39748	KP143732
<i>Karschia cezannei</i>	Ertz 19186 [T]	NG060323
<i>Karschia talcophila</i>	Ertz 16749	KP456155
<i>Labrocarpon canariense</i>	Ertz 16907	KP456158
<i>Lembosia abaxialis</i>	VIC 42825 [T]	NG060317
<i>Lembosia albersii</i>	MFLU 13-0377	KM386982
<i>Lembosia brigadeirensis</i>	VIC 44208	MF664531
<i>Lembosia minusopis</i>	MFLU 19-1225 [T]	MN563123
<i>Lembosia syzygii</i>	MFLU 13-0633	MG844185
<i>Lembosia xyliae</i>	MFLU 14-0004 [T]	NG059589
<i>Melaspilella proximella</i>	G.M. 2015-04-29	KY654747
<i>Melaspileopsis diplasiospora</i>	Ertz 16624	KP456165
<i>Parmularia styracis</i>	VIC 42447 [T]	NG060137
<i>Prillieuxina baccharidincola</i>	VIC 42817	KP143735
<i>Stictographa lentiginosa</i>	Ertz 17570	KP456170

[T] = Ex-types and authentic strains. Newly generated sequences are indicated in **bold**.

DNA extraction, PCR amplification, sequencing

Genomic DNA was extracted directly from fresh thyrlothecia using a Forensic Genomic DNA Extraction Kit.

The total volume of PCR mixture (25 µL) contained double distilled water (ddH₂O) (11 µL), 2× QInKe PCR Master Mix (11 µL), DNA template (1 µL) and 10 µM of each primer (1 µL). The complete 28S large subunit rDNA (LSU) gene was amplified using LR0R/LR5 primers (Vilgalys & Hester 1990). PCR amplification conditions consisted of an initial denaturation step of 5 min at 94 °C and final extension step of 10 minutes at 72 °C. For the LSU amplification, the 37 cycles consisted of denaturation at 94 °C for 1 minute, annealing at 54 °C for 50 seconds and elongation at 72 °C for 1 minute. PCR products were viewed on 1% agarose electrophoresis gels, stained with ethidium bromide (Thambugala & al. 2015). The amplified PCR products were sent to QInke, a commercial sequencing provider in Kunming, China.

Sequence alignment & phylogenetic analyses

Newly generated sequences were assembled using Seqman and subjected to the standard BLAST search (<https://blast.ncbi.nlm.nih.gov>) to identify the closest matches in GenBank. The accession numbers of taxa used in our analyses are shown in TABLE 1. Sequences (LSU) were aligned using MAFFT v. 6.864b (Katoh & al. 2017) and manually adjusted in BioEdit v. 7.0 (Hall 2004).

Maximum likelihood analysis was performed using RAxML on XSEDE in CIPRES (Miller & al. 2010, Silvestro & Michalak 2012). The optimal ML tree was obtained with 1000 separate runs under the GTR+GAMMA substitution model resulted from model tests. Bayesian inference (Larget & Simon 1999) was performed using the MrBayes 3.2.2 on XSEDE tool in CIPRES (Ronquist & al. 2012). Posterior probabilities (PP) were obtained from Markov chain Monte Carlo sampling (MCMC) (Rannala & Yang 1996, Ronquist & al. 2012) when the average standard deviation of split frequencies fell below 0.01. MCMC chains were run from random trees for 1,000,000 generations and sampled every 100 generations with the burn-in value of 25%. The remaining trees were used to calculate PP values. All trees were visualized in FigTree v1.4.0 (Rambaut 2012) and the final layout was done with Microsoft PowerPoint. The final alignment and trees were registered in TreeBASE (2019) under the submission ID: 25248

Phylogenetic conclusions

LSU sequence data of representative families comprised 39 strains, including two outgroup strains of *Parmularia styrcis* Lév. (VIC42447, VIC42450). The alignment contained 32,916 characters (LSU: 1-844) including alignment gaps. The best scoring RAxML tree was selected to represent the relationships among taxa with a final likelihood value of -5964.8722. The matrix had distinct alignment patterns with 12.01% undetermined characters or gaps. Estimated base frequencies were:

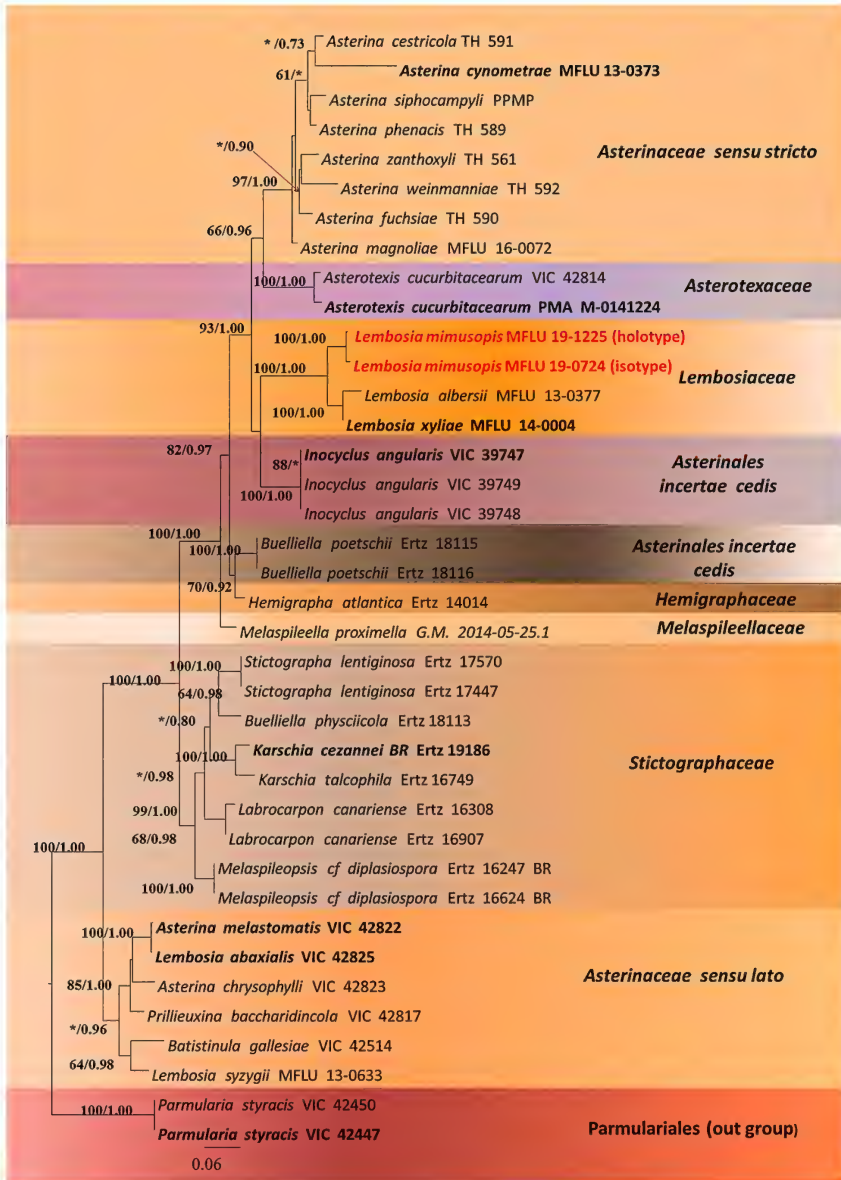


FIGURE 1. Phylogram of *Lembosia* and related genera, generated from maximum likelihood (RAxML) based on LSU sequence data. ML bootstrap support ($\geq 60\%$) and Bayesian posterior probability (≥ 0.80) are indicated above the branches as ML/PP. The tree is rooted in *Parmularia styrcis* (VIC 42447, VIC 42450). Ex-type strains are in bold and the newly generated sequences are in red.

A = 0.234839, C = 0.253073, G = 0.326282, T = 0.185806; substitution rates AC = 0.902460, AG = 2.658802, AT = 0.629941, CG = 1.176560, CT = 8.729825, GT = 1.000000; gamma distribution shape parameter α = 0.3196 (Fig. 1).

Lembosia albersii, *L. xylicae*, and our new taxon clustered with high statistical support (100% ML, 1 BYPP), forming a sister clade to three strains of *Inocyclus angularis* Guatim. & R.W. Barreto (VIC 39747, VIC 39748, VIC 39749). The new taxon is clearly distinct from the two other species in the clade with high statistical support. However, *L. abaxialis*, *L. brigadeirensis*, and *L. syzygii* clustered with species in *Asterina* sensu lato.

Taxonomy

Lembosia mimusopis Marasinghe, Daya., Maharachch., Hongsanan & K.D. Hyde, sp. nov.

FIG 2

IF 556875

Differs from *Lembosia bomjardinensis* in having ascospores that are sheathed when immature.

TYPE: Thailand, Chiang Rai, MFU Botanical Gardens, on living leaves of *Mimusops elengi* L. (*Sapotaceae*), 19 December 2018, M.W.D. Sandamali BSM-2019a (Holotype, MFLU 19-1225; GenBank MN563123. Isotype, MFLU 19-0724; GenBank MN565982).

ETYMOLOGY: referring to the host genus *Mimusops* from which the fungus was collected.

COLONIES hypophyllous on *Mimusops elengi* leaves forming blackened circular to irregular areas, single to confluent. HYPHAE 3.2–5 μ m diam. ($x = 4.5 \mu$ m, $n = 10$), superficial, brown, straight to undulate, branching opposite to irregular at acute to wide angles, reticulate. APPRESSORIA 4–5.5 μ m diam. ($x = 4.5 \mu$ m, $n = 10$), unicellular, brown, irregular, opposite to alternate.

SEXUAL MORPH: THYRIOTHECIA 350–500 $\times$ 350–450 μ m ($x = 451 \times 393 \mu$ m, $n = 6$), scattered, sub-dense, initially rounded, elongated at maturity, longitudinally dehiscent at the centre, margin crenate to fimbriate, borne on the surface of mycelium. PSEUDOPARAPHYSES not observed. ASCI 55–65 $\times$ 45–55 μ m ($x = 60 \times 48 \mu$ m, $n = 10$), 8-spored, bitunicate, sub-globose to globose, apical region of asci usually thick, opaque, ocular chamber not observed, not staining blue in IKI. ASCOSPORES 25–30 $\times$ 8–11 μ m ($x = 28 \times 9 \mu$ m, $n = 10$), overlapping 4–5-seriate, oblong to obovoid, with broad to narrowly rounded ends, lower cell slightly longer and narrower, hyaline, brown when mature, covered with mucilaginous sheath when immature, 1-septate, constricted at the septum, verruculose.

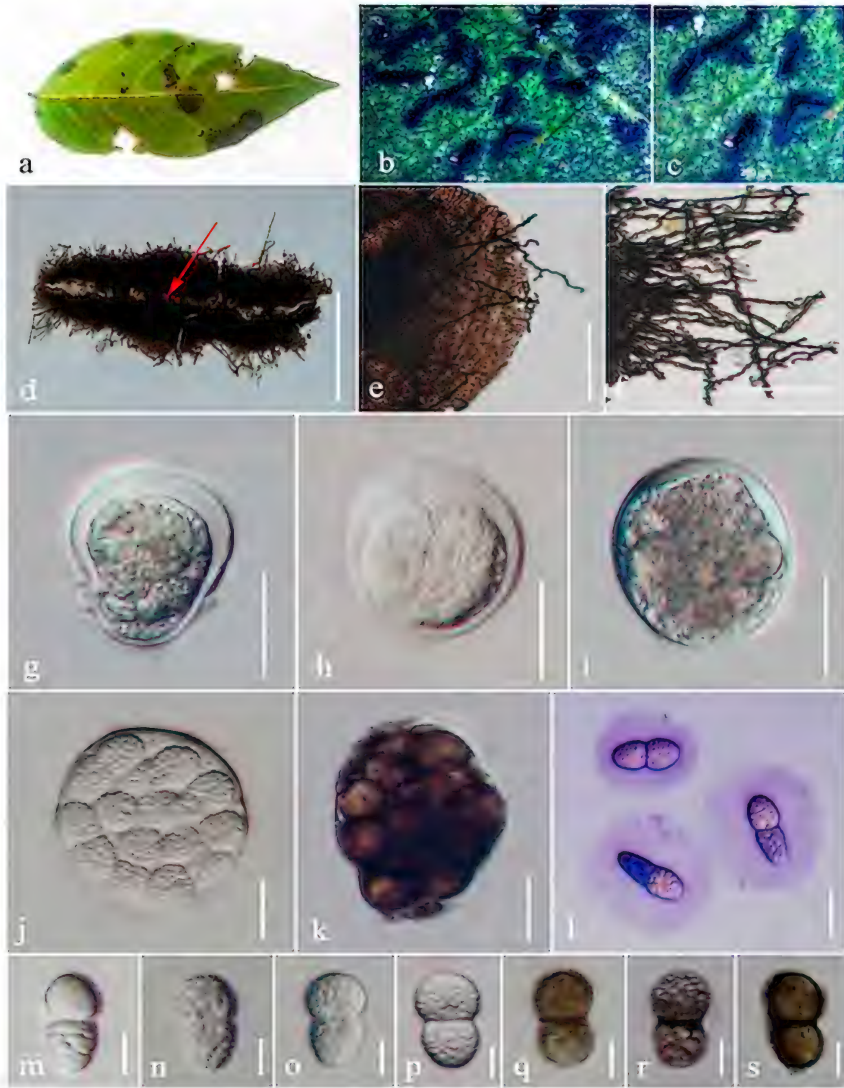


FIGURE 2. *Lembosia mimusopis* (holotype, MFLU 19-1225): a. Leaf sample; b, c. Thyriothecia on host substrate; d. Squash mount of thyriothecium showing longitudinal fissure (red arrow); e. Upper wall of thyriothecium; f. Hyphae with appressoria; g–j. Immature asci; k. Mature ascus; l. Ascospores with mucilaginous sheath stained with blue ink; m–s. Immature to mature ascospores. Scale bars: d–f = 100 μ m; g–k = 20 μ m; l–s = 10 μ m.

ASEXUAL MORPH: Undetermined.

COMMENTS: *Lembosia mimusopis* (FoF06234) is morphologically similar to *L. bomjardinensis* (Bat.) Hongsanan & K.D. Hyde [$\equiv$ *Yamamotoa bomjardinensis* Bat.] in its globose to subglobose, apedicellate asci with a thick hyaline apical region and oblong to obovoid, 1-septate, hyaline to brown ascospores with a verruculose wall. However, *L. mimusopis* is distinguished by having sheathed ascospores when immature; and in its sapotaceous host in contrast to the proteaceous host of *L. bomjardinensis*. Attempts to isolate *L. mimusopis* onto potato dextrose agar and malt extract agar were unsuccessful so that the DNA was extracted directly from fruiting bodies.

Discussion

Lembosia mimusopis represents the first record of a *Lembosia* species from *Mimusops* (Sapotaceae). However, *L. sapotacearum* Mibey was recorded from Sapotaceae in East Africa (Mibey & Hawksworth 1997). *Lembosia* species are also recorded from other host families (Araucariaceae, Asparagaceae, Dipterocarpaceae, Ebenaceae, Lauraceae, Myrtaceae, Proteaceae, Orchidaceae; Index Fungorum 2019). A comprehensive comparison of morphology within this genus is complicated by the lack of good illustrations and descriptions of previously recorded species. Host-specificity of *Lembosia* species has not been phylogenetically studied (Hongsanan & al. 2014). Hongsanan & al. (2014) synonymized *Trichamelia* Bat., *Viegasia* Bat., and *Yamamotoa* Bat. under *Lembosia*. Dai & al. (2018) pointed out the necessity of recollecting the type species, *L. tenella*, to obtain molecular data to establish its placement within *Asterinales*. In agreement with the results reported by Dai & al. (2018), our study also shows that four *Lembosia* species form a sister clade with *Inocyclus* in *Asterinales*.

Acknowledgments

The authors extend their appreciation to the Researchers supporting project number (RSP-2019/56) King Saud University, Riyadh, Saudi Arabia. We would like to thank the National Natural Science Foundation of China for supporting the project Biodiversity, Taxonomy, Phylogeny, Evolution and Phytogeography of Phytopathogens in *Dothideomycetes* from Southern China (Project number: 31950410548) for funding this research. The Thailand Research fund is thanked for the grant “Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion grant number: RDG6130001” and Thailand Science Research and Innovation (TSRI) grant “Macrofungi diversity research from the Lancang-Mekong Watershed and Surrounding areas (grant no. DBG6280009). Prof. K.D. Hyde thanks Chiang Mai University for the award of Visiting Professorship.

The authors thank Prof. D. Jayarama Bhat (Curca, Goa Velha-403108, India) and Dr. Eric H.C. McKenzie (Manaaki Whenua Landcare Research, Auckland, New Zealand) for presubmission review. Prof. Mibey and Dr. Shaun Pennycook are thanked for valuable suggestions and for nomenclatural advice.

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